

We claim:

1                    1.     A method for measuring the presence of oncogenic activity of  
2     intracellular chemical reactions in a cell or cells comprising:

3                    providing substrate molecules for an oncoprotein containing a label, the  
4                    labeled substrate molecules corresponding to chemical reactions  
5                    whose activity is to be measured;

6                    disposing said substrate molecules within said cell or cells;

7                    allowing said substrate molecules within said cell or cells to take part in  
8                    the chemical reaction to produce altered substrate molecules;

9                    liberating said substrate molecules and said altered substrate molecules  
10                    from the single cell;

11                    detecting the label to identify the substrate molecules and/or the altered  
12                    substrate molecules from said cell or cells; and

13                    determining the presence of said chemical reaction from the presence of  
14                    modified substrate.

1                    2.     The method of claim 1 further comprising quantifying the amounts  
2     of detected altered substrate molecules and/or detected unaltered substrate molecules.

1                    3.     The method of claim 1 wherein said intracellular chemical reaction  
2     in said cell comprises enzyme catalysis by a kinase.

1                   4.     The method of claim 1 wherein said altered substrate molecules  
2     exhibit a change in chemical structure as compared with the unaltered substrate  
3     molecules.

1                   5.     The method of claim 4, wherein separating said unaltered substrate  
2     molecules and said altered substrate molecules comprises electrophoresis.

1                   6.     The method of claim 2 wherein quantifying the amounts of detected  
2     altered substrate molecules and detected unaltered substrate molecules comprises  
3     detection of the label by fluorescence following separation by electrophoresis.

1                   7.     The method of claim 1 wherein disposing said unaltered substrate  
2     molecules within said cell or cells comprises using a naturally occurring substrate  
3     molecule within said cell or cells, inducing said substrate molecule to be produced  
4     within said cell or cells, or introducing said substrate molecule into said cell or cells from  
5     outside said cell or cells.

1                   8.     The method of claim 7 wherein introducing said unaltered substrate  
2     molecules into said cell or cells from outside said cell or cells comprises microinjecting,  
3     electroporating, optoporating, vesicle fusing, pinocytic loading, or associating said  
4     substrate molecules with membrane permeant peptides.

1           9.     The method of claim 1 further comprising stimulating said cell or  
2 cells while said unaltered substrate molecules are intracellularly present prior to  
3 liberating said unaltered substrate molecules and said altered substrate molecules from  
4 the single cell or cells.

1           10.    The method of claim 9 further comprising comparing activity of said  
2 chemical reaction with a similar activity determined from said single cell or cells that has  
3 not been stimulated.

1           11.    The method of claim 1, wherein liberating said unaltered substrate  
2 molecules and said altered substrate molecules from the cell or cells comprises  
3 chemical disruption of said single cell or cells, mechanical disruption of said single cell  
4 or cells, or by electrical disruption, or by a combination thereof.

1           12.    The method of claim 1, wherein the label is selected from a group  
2 consisting of fluorescent labels, isotopes, labels exhibiting optical absorption, and  
3 electron spin resonance labels.

1           13.    The method of claim 1 wherein the substrate molecules are  
2 polymers.

1           14.    The method of claim 13 wherein the polymers are selected from a  
2 group consisting of peptides, polysaccharide, and nucleic acids.

1                   15.    The method of claim 14 wherein said polymers are modified with a  
2   fluorescent label.

1                   16.    The method of claim 14 wherein said peptides are substrates for a  
2   kinase that alters said modified peptides by the addition of a phosphate moiety to a  
3   particular amino acid within each peptide.

1                   17.    The method of claim 16, wherein said peptide has been modified  
2   by covalent addition of a fluorescent group.

1                   18.    The method of claim 1, said substrate molecules comprise  
2   carbohydrates, phospholipids, entire proteins, or organic compounds not ordinarily  
3   found within the cell.

1                   19.    The method of claim 1 wherein detecting the label comprises  
2   performing voltammetry or mass spectrometry.

1                   20.    The method of claim 1 further comprising simultaneously  
2   performing each of said steps with a plurality of different substrate molecules, each  
3   reporting on a specific chemical reaction within said cell or cells.

1           21.    A method for measuring oncogenic activity of a chemical reaction  
2 in a minute volume of tens of pl or less comprising:

3           providing substrate molecules for an oncoprotein containing a label;

4           disposing said substrate molecule into said minute volume where said

5           chemical reactions occurs producing altered substrate molecules

6           within said minute volume;

7           terminating said chemical reactions;

8           detecting the label to identify unaltered substrate molecules and/or the

9           altered substrate molecules to determine activity of the chemical

10          reaction corresponding to the oncoprotein.

1           22.    The method of claim 21 further comprising quantifying changes in  
2 the amounts of the unaltered substrate molecules and/or the altered substrate  
3 molecules.

1           23. An apparatus for measuring an activity of oncogenic chemical  
2 reactions of intracellular molecules comprising:

3           means for disposing labeled substrate molecules into said cell or cells to

4           form labeled altered substrate molecules therein;

5           means for liberating said substrate and/or altered substrate molecules

6           from said cell or cells;

7           means for separating said substrate and/or altered substrate molecules

8           from each other;

9           means for detecting said unaltered substrate molecules and said altered

10          substrate molecules from a cell or cells before any substantial

11          alteration of said unaltered substrate molecules and said altered

12          substrate molecules has occurred.

1           24. The apparatus of claim 23 further comprising means for quantifying  
2 changes in the amounts of said unaltered substrate molecules and/or said altered  
3 substrate molecules.

1           25. The apparatus of claim 23 wherein said means for detecting  
2 detects enzyme catalysis by a kinase.

1           26. The apparatus of claim 23 wherein said means for detecting  
2 detects a change in electrophoretic mobility of said unaltered substrate molecules  
3 versus said altered substrate molecules.

1           27.    The apparatus of claim 23 wherein said means for separating  
2 comprises capillary electrophoresis.

1           28.    The apparatus of claim 24 wherein said means for quantifying  
2 changes in the amounts of said unaltered substrate molecules and/or said altered  
3 substrate molecules comprises means for quantifying fluorescence of said unaltered  
4 substrate molecules and/or said altered substrate molecules following separation of  
5 said unaltered substrate molecules and said altered substrate molecules by capillary  
6 electrophoresis.

1           29.    The apparatus of claim 23 wherein said means for disposing  
2 comprises means for inducing said substrate molecules to be produced within said cell  
3 or cells, or means for introducing said substrate molecule into said cell or cells from  
4 outside said cell or cells.

1           30.    The apparatus of claim 29 wherein said means for introducing said  
2 substrate molecules into said cell or cells from outside said cell or cells comprises  
3 means for microinjecting, means for electroporating, means for optoporating, means for  
4 vesicle fusing, means for pinocytic loading, or means for associating said substrate  
5 molecules with membrane permeant peptides.

1           31. The apparatus of claim 23 wherein said means for disposing said  
2 substrate molecules comprises means for providing said substrate molecules from  
3 naturally occurring compounds or synthetically derived compounds.

1           32. The apparatus of claim 23 further comprising means for stimulating  
2 said cell or cells while said substrate molecules are present intracellularly prior to  
3 detecting said unaltered substrate molecules and said altered substrate molecules.

1           33. The apparatus of claim 32 wherein said means for detecting  
2 comprises means for obtaining the contents of said cell or cells, and means for  
3 separating part or all of said contents by capillary electrophoresis

1           34. The apparatus of claim 33, wherein said means for obtaining the  
2 contents comprises chemical means for disruption, physical means for disruption,  
3 electrical means for disruption, or a combination thereof.

1           35. The apparatus of claim 23 wherein said means for disposing  
2 provides substrate molecules that correspond to oncogenic intracellular chemical  
3 reactions.

1           36. The apparatus of claim 35 wherein said means for disposing  
2 provides substrate molecules which are fluorescent.



1           37. The apparatus of claim 36 wherein said means for disposing  
2 provides substrate molecules which are modified peptides.

1           38. The apparatus of claim 37, wherein said modified peptides are  
2 substrates for a kinase that alters said modified peptides by the addition of a phosphate  
3 moiety to a particular amino acid within each said peptide.

1           39. The apparatus of claim 37 wherein said peptides have been  
2 modified by covalent addition of a fluorescent group to allow detection by fluorescence.

1           40. The apparatus of claim 23 wherein said means for disposing  
2 provides substrate molecules comprising nucleic acids, carbohydrates, phospholipids,  
3 entire proteins, or compounds not ordinarily found within cells.

1           41. The apparatus of claim 23 wherein said means for detecting  
2 comprises means for performing voltammetry or means for mass spectrometry on said  
3 unaltered substrate molecules and said altered substrate molecules.

1           42. The apparatus of claim 23 further comprising means for  
2 simultaneously disposing and detecting a plurality of different substrate molecules,  
3 each different substrate molecule reporting on chemical reactions within said cell or  
4 cells.

1           43. An apparatus for measuring an activity of chemical reactions of  
2 molecules in a minute volume of the order of 100 pl or less comprising:

3           means for disposing substrate molecules having a label into said minute  
4           volume for oncogenic chemical reactions to occur producing  
5           altered substrate molecules; and

6           means for detecting the label to identify the unaltered substrate molecules  
7           and the altered substrate molecules to determine activity of the  
8           oncogenic chemical reaction.

1           44. The apparatus of claim 43 further comprising means for quantifying  
2 changes in the amounts of said unaltered substrate molecules and said altered  
3 substrate molecules.

1           45. An apparatus for measuring an activity of oncogenic intracellular  
2 chemical reactions of molecules in a cell or cells in which labeled substrate molecules  
3 have been disposed to allow for an in vivo reaction wherein labeled altered substrate  
4 molecules are formed, comprising:

5           a detector of said labeled unaltered substrate molecules and said labeled  
6           altered substrate molecules; and

7           a cell sampling device communicating with said detector, which cell  
8           sampling device extracts said unaltered substrate and altered  
9           substrate molecules from said cell, and which cell sampling device

10 collects and transfers said unaltered substrate and altered  
11 substrate molecules into said detector before any further  
12 substantial alteration occurs.

1 46. The apparatus of claim 45 further comprising a data processor  
2 coupled to said detector to quantify changes in the amounts of said unaltered substrate  
3 molecules and said altered substrate molecules.

1 47. The apparatus of claim 23 further comprising a data processor  
2 coupled to said detector to quantify changes in the amounts of said unaltered substrate  
3 molecules and said altered substrate molecules.

1 48. The apparatus of claim 43 further comprising a data processor  
2 coupled to said detector to quantify changes in the amounts of said unaltered substrate  
3 molecules and said altered substrate molecules.

5 49. The method of claim 1 where oncogenic activity of intracellular  
chemical reactions in a cell is related to a bcr-abl oncoprotein and where said providing  
substrate molecules for a kinase provides substrates for bcr-abl tyrosine kinase.

50. The method of claim 21 where said oncogenic activity is related to  
a bcr-abl oncoprotein and where providing substrate molecules for a kinase comprises  
providing substrates molecules for bcr-abl tyrosine kinase.

51. The apparatus of claim 23 where said activity of oncogenic chemical reactions of intracellular molecules is related to a bcr-abl oncoprotein, where said means for disposing labeled substrate molecules into said cell disposed substrate molecules for bcr-abl tyrosine kinase, and where said means for detecting said  
5 unaltered substrate molecules and said altered substrate molecules detects phosphorylated substrate molecules for bcr-abl tyrosine kinase.

52. The apparatus of claim 43 where said activity of oncogenic intracellular chemical reactions is related to a bcr-abl oncoprotein, where said detector of said labeled unaltered substrate molecules and said labeled altered substrate  
10 molecules detects phosphorylated and/or nonphosphorylated substrate molecules for bcr-abl tyrosine kinase.

53. The apparatus of claim 45 where said activity of oncogenic chemical reactions of intracellular molecules is related to a bcr-abl oncoprotein, where said means for disposing labeled substrate molecules into said cell disposed substrates  
15 molecules for bcr-abl tyrosine kinase, and where said means for detecting said unaltered substrate molecules and said altered substrate molecules detects phosphorylated and/or nonphosphorylated substrate molecules for bcr-abl tyrosine kinase.